U.S. DEPARTMENT OF COMMERCE
National Technical Information Service
PB80-129901

Isozyme Profiles of Lactic Dehydrogenase and Creatine Phosphokinase in Neonatal Mouse Hearts

(U.S.) Health Effects Research Lab, Research Triangle Park, NC

1978

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3.
EPA-600/J-78-159	JOURNAL ARTICLE	PB80-129901

4. TITLE AND SUBTITLE	5. REPORT DATE
Isozyme Profiles of Lactic Dehydrogenase and Creatine Phosphokinase in Neonatal Mouse Hearts	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) K. D. Courtney, M. T. Ebron	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Toxicology Division Health Effects Research Laboratory U. S. Environmental Protection Agency Research Triangle Park, NC 27711	10. PROGRAM ELEMENT NO. 1EA615 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory RTP, NC Office of Research and Development U. S. Environmental Protection Agency Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA 600/11

15. SUPPLEMENTARY NOTES

Published in: Biology of the Neonate 34:203-208, 1978

16. ABSTRACT

Isozyme profiles of lactic dehydrogenase (LDH) and creatine phosphokinase (CPK) were determined in cardiac tissue of mice during postnatal development. LDH isozymes 1 and 5 showed a definite developmental change, achieving the adult values by 20 days of age, while the other three isozymes showed no change. During the first 2 postnatal weeks, a developmental change was seen in the activities of the "mito" and MB isozymes of CPK, with the adult profile being established by day 20. CPK isozymes MM and BB did not change during this interval.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Profiles Hearts	Lactic Dehydrogenase Creatine Phosphokinase	06A,C,T

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	8
	20. SECURITY CLASS (This page)	22. PRICE
	UNCLASSIFIED	A02 - A01

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA-600/J-78-159
JOURNAL ARTICLE

Biology of the Neonate
Editor-in-Chief: A. Minkowski, Paris

Publishers: S. Karger, Basel
Reprint (Printed in Switzerland)

Biol. Neonate *34:* 203–208 (1978)

Isozyme Profiles of Lactic Dehydrogenase and Creatine Phosphokinase in Neonatal Mouse Hearts

K.D. Courtney and M.T. Ebron

Health Effects Research Laboratory, Environmental Toxicology Division, US Environmental Protection Agency, Research Triangle Park, N.C.

Key Words. LDH isozymes · CPK isozymes · Cardiac · Mouse · Postnatal

Abstract. Isozyme profiles of lactic dehydrogenase (LDH) and creatine phosphokinase (CPK) were determined in cardiac tissue of mice during postnatal development. LDH isozymes 1 and 5 showed a definite developmental change, achieving the adult values by 20 days of age, while the other three isozymes showed no change. During the first 2 postnatal weeks, a developmental change was seen in the activities of the 'mito' and MB isozymes of CPK, with the adult profile being established by day 20. CPK isozymes MM and BB did not change during this interval.

Introduction

Isozymes are now widely recognized for their importance in developmental biology and medicine (3, 4, 10, 12, 14, 15). That isozyme profiles permit the investigation of functional changes which occur during cellular differentiation (13) has been well demonstrated during embryogenesis, but few studies had employed the neonate or the time interval from birth to adult life even though many developmental changes occur during this time. In many teratology studies, the fetal and neonatal hearts are seldom evaluated beyond a gross anatomical inspection due to the small size of this organ; therefore, the isozyme profiles of lactic dehydrogenase (LDH) and creatine phosphokinase (CPK) were established as quantitative methods of evaluating postnatal cardiac development. LDH and CPK isozyme profiles are currently being used clinically as indicators of disease or malfunction of cardiac muscle (2, 7, 8).

Methods

Seven parameters were determined for each mouse: body weight, heart weight, total LDH activity, total CPK activity, LDH isozyme profile, CPK isozyme profile, and protein concentration of the 500 *g* supernate. All results are presented as the mean ± SE.

Experimental Animals

Mice of the CD-1 strain were obtained from Charles River Laboratories, Inc., Wilmington, Mass. with known insemination dates. At the expected time of parturition, the mice were observed daily at 9:00 a.m. and litters born during the preceding 24 h were

noted as 1 day old. For the biochemical determinations, 1 or 2 mice were randomly selected from the litters.

Supernate Preparation

The mice were weighed and decapitated. The heart was removed, sliced open and washed in 0.85% saline to remove all blood. Organs were blotted, weighed, and homogenized in 20 vol of 0.1 *M* sodium phosphate buffer, pH 7.4 at room temperature. The homogenate was centrifuged at 500 *g* for 30 min in a refrigerated centrifuge.

Total LDH and CPK Activities

The total LDH and CPK activities were determined spectrophotometrically using Kit No. 500 and 520, respectively, of Sigma Chemical Co., St. Louis, Mo.

Isozyme Determination

The isozymes were separated by electrophoresis using the Beckman Microzone Electrophoresis system (Beckman Instruments, Palo Alto, Calif.) with Beckman cellulose acetate membranes and Gelman high resolution buffer, pH 8.8, ionic strength 0.06 *M* (Gelman Instruments Co., Ann Arbor, Mich.). The cellulose acetate strips were saturated with buffer before positioning in the cell. Using the Beckman applicator, 75 µl of homogenate were applied, and electrophoresis carried out for 30 min at 250 V at room temperature for both LDH and CPK.

The LDH isozymes were visualized by development with the Iso-form LDH kit from Dade Division, American Hospital Supply Co., Miami, Fla. The CPK isozymes were visualized using both the Iso-form LDH kit of Dade Division and the CPK tablets of Smith-Klein Inst., Inc., Sunnyvale, Calif. The CPK tablets were dissolved (20 tablets/30 ml dist. H_2O) and 5.5 ml of the solution added to the dissolved Noble agar before applying the acetate strip (16). The CPK cellulose acetate strips were incubated at 37 °C for 30 min to develop the color while the LDH cellulose acetate strips were developed at room temperature.

The acetate strips were scanned with a Beckman Recording Densitometer and R-111 Digital Integrator, which determined relative peak heights as percents.

Protein Determination

The protein content of a 25-µl aliquot of the supernate was determined by the method of *Murphy and Kies* (11).

Results and Discussion

Presented in table I are the ages of the mice, number of mice, body weights, heart weights and protein concentration of the supernate. The body and heart weights showed a normal growth pattern from 7 days of age to adult. An adult in this study was 90–120 days old.

The total activities of LDH and CPK are presented in the bottom graph of figure 1. LDH activity showed an increase from days 7 to adult, with a slight decrease in activity on days 12, 18, and 19. Comparable decreases were also seen in the total CPK activity shown on the same graph. Days 12 and 18–19 are postnatal event markers, signaling the onset of eye opening and weaning, respectively. These events are probably coincident with the changes in enzyme activity and not the cause. From day 20 to adult, the total activities of both LDH and CPK remain quite constant.

LDH can be resolved into five isozymes by electrophoresis. Each isozyme is a tetramer of 4 protein subunits. LDH-1 is composed of 4 units of H (heart) and LDH-5 is composed of 4 units of M (muscle). Isozymes 2, 3, and 4 are represented as HHHM, HHMM, and HMMM, respectively. The embryonic form of cardiac LDH is mainly LDH-5 with a gradual shift during development to LDH-1.

The developmental changes in the LDH isozyme profile are shown in the upper graph of figure 1 and the means with standard errors are in table II. LDH-1 was relatively low in activity on day 7 and steadily increased to adult values by day 20. LDH-5 was relatively high in activity and remained high until day 14 when it decreased, reaching adult values by day 20. Isozymes 2, 3, and 4 remained fairly constant throughout postnatal development. This developmental profile is similar to that seen in the neonatal rat and human (1, 9).

Table I. Body weight, heart weight, and total protein of 500 *g* supernate of CD-1 mice from 7 days of age to adult

Age, days	Number of mice	Body weight, g		Heart weight, mg		mg supernate protein/heart	
		mean	SE	mean	SE	mean	SE
7	24	3.6	0.08	20.0	0.80	106.3	8.5
8	23	4.0	0.08	21.2	0.80	120.0	5.5
9	23	4.5	0.10	24.0	0.80	121.6	6.8
10	14	5.2	0.10	26.6	0.90	108.4	7.8
11	13	5.7	0.20	36.7	2.20	152.8	11.0
12	18	7.0	0.30	39.8	1.70	160.9	9.9
13	28	7.2	0.30	38.7	1.00	172.5	14.7
14	12	7.3	0.40	39.1	1.70	187.5	14.5
15	21	7.5	0.30	37.7	1.30	217.5	12.2
16	16	8.2	0.30	40.5	1.30	246.4	15.3
17	15	8.3	0.30	41.0	1.20	246.2	18.1
18	14	8.5	0.20	46.4	1.30	285.9	18.7
19	16	8.9	0.30	45.7	1.40	300.3	16.1
20	16	9.3	0.40	48.5	1.80	381.9	16.6
21	8	14.1	0.70	65.1	2.70	374.1	30.5
22	3	12.1	0.90	56.0	3.10	343.7	22.5
30	8	25.6	1.30	97.6	4.70	515.6	48.6
50	3	30.4	1.50	119.0	3.90	770.7	43.1
90–120	17	38.2	1.10	148.0	3.90	917.3	49.5

CPK is composed of four isozymes and is generally regarded as a dimeric structure with an M (muscle) and a B (brain) subunit. The isozymes are MM, BB, MB, and a mitochondrial subunit commonly referred to as the 'mito' isozyme.

The developmental changes in the CPK isozymes are shown in the middle graph of figure 1 and the means with standard errors are presented in table III. Of particular note with the CPK isozymes was the appearance of the 'mito' isozyme. From day 1 through day 5 (not shown on graph) it was not detectable. From day 7 to day 17 there was a slow and steady increase in the 'mito' isozyme, and during this time a steady decrease in the MB isozyme. About day 20 the relative activities of MB and 'mito' displayed the adult profile in that the 'mito' isozyme had a greater activity than the MB isozyme. The MM isozyme was fairly constant during development.

Hall and DeLuca (5) reported that the BB isozyme of CPK in the neonatal mouse heart disappeared by day 18. He subsequently determined the decline could be due to insufficient sulfhydryl reagent (6). In our studies, the BB isozyme was always detectable with no indication of disappearing.

In summary, the total activities of LDH and CPK showed a general increase from postnatal day 7 to adulthood. The isozymes of both LDH and CPK showed a definite developmental

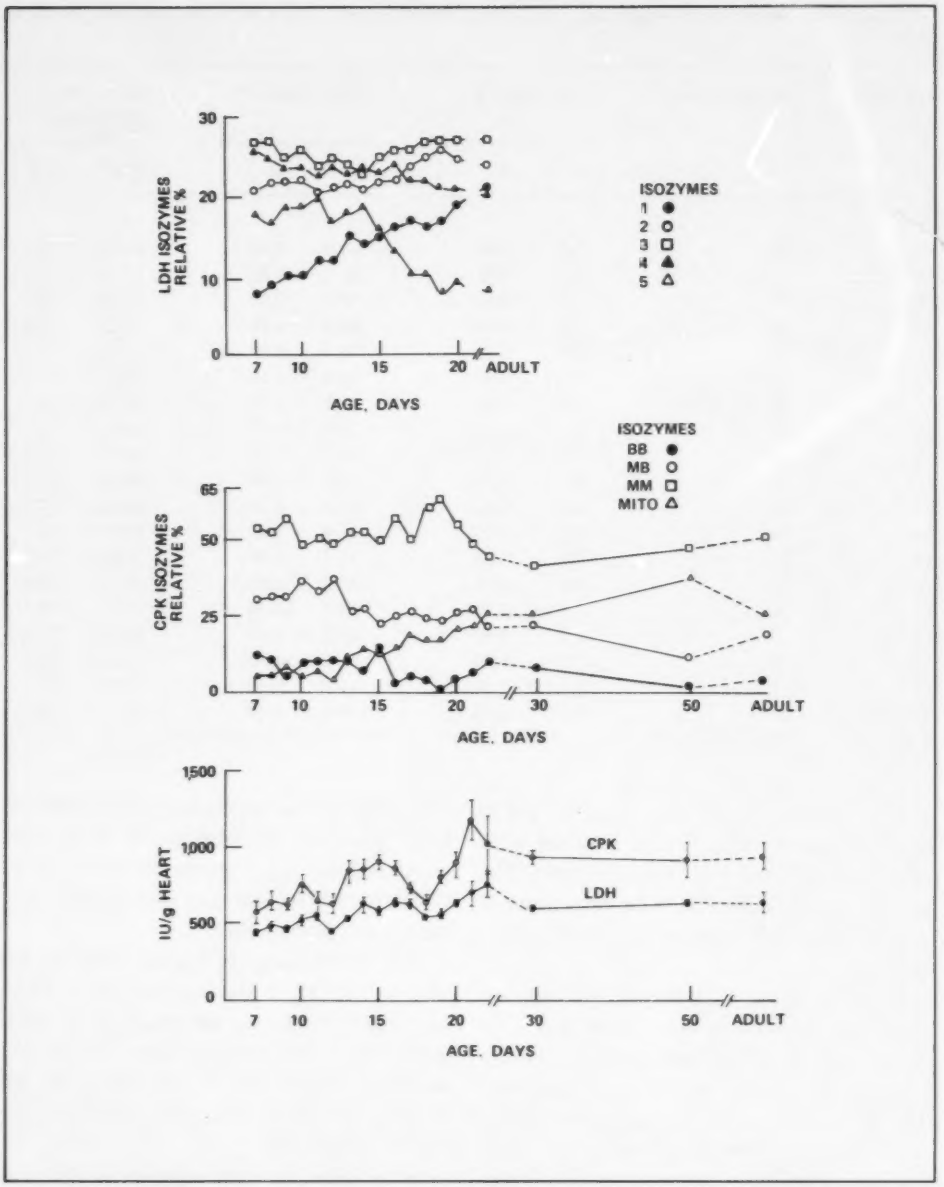

Fig. 1. The activities of the LDH and CPK isozymes of mouse cardiac tissue presented as relative percents of total activity are shown in the two upper graphs. The total activities of LDH and CPK, IU/g wet weight of heart, are shown in the bottom graph.

Table II. Isozymes of LDH in cardiac tissue of CD-1 mice during postnatal development; results are average relative percents ± SE

Age, days	number of mice	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
7	21	8.1 ± 0.5	21.1 ± 0.2	27.1 ± 0.6	25.7 ± 0.4	18.1 ± 0.4
8	23	9.3 ± 0.4	21.8 ± 0.3	26.6 ± 0.3	24.9 ± 0.3	17.4 ± 0.7
9	23	9.9 ± 0.5	21.7 ± 0.4	25.3 ± 0.4	23.8 ± 0.2	19.3 ± 0.5
10	14	9.6 ± 1.2	21.7 ± 0.4	25.9 ± 0.4	23.9 ± 0.4	18.9 ± 1.0
11	13	12.1 ± 0.8	20.9 ± 0.4	23.8 ± 0.5	23.2 ± 0.4	20.0 ± 0.8
12	18	12.0 ± 0.5	21.3 ± 0.3	25.2 ± 0.4	24.0 ± 0.4	17.4 ± 0.6
13	22	14.5 ± 0.5	21.8 ± 0.3	23.5 ± 0.4	22.5 ± 0.2	17.7 ± 0.5
14	12	14.4 ± 0.4	21.1 ± 0.5	23.4 ± 0.3	22.6 ± 0.1	18.6 ± 0.9
15	19	14.5 ± 0.7	22.3 ± 0.3	25.1 ± 0.4	22.8 ± 0.2	15.4 ± 0.6
16	16	15.5 ± 0.3	22.4 ± 0.3	25.5 ± 0.4	23.7 ± 0.5	12.9 ± 0.8
17	15	17.0 ± 0.4	24.1 ± 0.3	26.4 ± 0.6	22.4 ± 0.3	10.2 ± 1.0
18	14	16.4 ± 0.6	24.7 ± 0.3	27.1 ± 0.2	22.1 ± 0.4	9.6 ± 0.5
19	16	17.2 ± 0.5	25.9 ± 0.7	26.6 ± 0.3	20.8 ± 0.5	8.0 ± 0.9
20	16	18.5 ± 0.5	25.3 ± 0.3	26.5 ± 0.2	21.0 ± 0.4	8.7 ± 0.4
30	8	18.6 ± 0.4	23.0 ± 0.9	24.3 ± 0.9	20.6 ± 0.4	12.1 ± 1.5
50	3	20.1 ± 0.4	24.3 ± 0.4	24.9 ± 0.5	18.8 ± 0.2	11.8 ± 0.2
90–120	17	20.8 ± 1.1	24.5 ± 1.3	26.0 ± 0.4	17.8 ± 0.6	8.5 ± 0.8

Table III. Isozymes of CPK in cardiac tissue of CD-1 mice during postnatal development; results are average relative percents ± SE

Age, days	Number of mice	CPK-BB	CPK-MB	CPK-MM	CPK-mito
7	24	12.0 ± 1.4	30.4 ± 1.4	52.6 ± 2.2	5.1 ± 0.8
8	23	11.3 ± 0.7	30.9 ± 0.5	52.2 ± 1.1	5.0 ± 1.0
9	23	5.0 ± 0.6	30.5 ± 1.0	57.3 ± 1.6	7.9 ± 1.4
10	14	9.3 ± 1.6	36.6 ± 1.7	47.8 ± 1.5	6.2 ± 1.3
11	12	9.7 ± 1.4	32.9 ± 2.0	50.4 ± 1.5	7.1 ± 1.4
12	11	11.0 ± 1.0	36.8 ± 2.2	47.9 ± 1.6	4.4 ± 0.9
13	22	9.9 ± 0.9	25.5 ± 0.6	52.5 ± 0.7	12.2 ± 0.9
14	11	6.8 ± 1.2	27.0 ± 0.8	52.0 ± 0.3	13.6 ± 0.5
15	19	13.8 ± 1.7	23.1 ± 0.7	49.3 ± 0.6	13.8 ± 0.8
16	16	3.4 ± 0.6	24.8 ± 0.3	57.1 ± 0.4	14.8 ± 0.6
17	15	5.8 ± 0.4	26.4 ± 0.3	48.5 ± 0.5	19.4 ± 0.4
18	14	4.3 ± 0.7	18.9 ± 0.8	60.0 ± 1.5	16.9 ± 1.0
19	16	0.7 ± 0.5	19.0 ± 0.8	62.9 ± 1.3	17.3 ± 1.2
20	16	3.8 ± 0.4	21.4 ± 0.4	54.0 ± 0.7	20.9 ± 1.0
30	8	7.6 ± 0.7	22.0 ± 1.5	44.1 ± 0.7	26.3 ± 1.7
50	3	2.3 ± 1.0	12.3 ± 1.9	47.3 ± 2.9	38.0 ± 1.4
90–120	16	4.0 ± 1.1	19.7 ± 1.7	48.6 ± 1.9	26.1 ± 1.9

pattern with adult profiles established by day 20. Currently, studies are under way in our laboratory using the developmental profiles as indicators of toxic effects from prenatal and postnatal administration of pesticides which is the subject of another report.

References

1 Blatt, W.F.; Blatteis, C.M., and Mager, M.: Tissue lactic dehydrogenase isozymes. Developmental patterns in the neonatal rat. Can. J. Biochem. *44:* 537–543 (1966).

2 Blomberg, A.J.; Kimber, W.D., and Burke, M.D.: Creatine kinase isoenzymes. Predictive value in the early diagnosis of acute myocardial infarction. Am. J. Med. *59:* 464–469 (1975).

3 Brinkworth, R.I. and Masters, C.J.: Ontogenetic characteristics of lactate dehydrogenase turnover in the mouse. Biochim. biophys. Acta *482:* 241–250 (1977).

4 Cornish, H.H.; Barth, M.L., and Dodson, V.N.: Isozyme profiles and protein patterns in specific organ damage. Toxic. appl. Pharmac. *16:* 411–423 (1970).

5 Hall, N. and DeLuca, M.: Developmental changes in creatine phosphokinase isoenzymes in neonatal mouse hearts. Biochem. biophys. Res. Commun. *66:* 988–994 (1975).

6 Hall, N. and DeLuca, M.: Electrophoretic separation and quantitation of creatine kinase isozymes. Analyt. Biochem. *76:* 561–567 (1976).

7 Hillis, L.D. and Braunwald, E.: Myocardial ischemia. Part II. New Engl. J. Med. *296:* 1034–1041 (1977).

8 Hillis, L.D. and Braunwald, E.: Myocardial ischemia. Part III. New Engl. J. Med. *296:* 1093–1096 (1977).

9 Mino, M.; Ueda, Y.; Fujitani, K.; Tatsuta, T., and Takai, T.: Enzymatic properties of lactate dehydrogenase isozymes in human fetal tissues. Sabco J. *1:* 121–129 (1965).

10 Monk, M. and Ansell, J.: Patterns of lactic dehydrogenase isozymes in mouse embryos over the implantation period *in vivo* and *in vitro*. J. Embryol. exp. Morph. *36:* 653–662 (1976).

11 Murphy, J.B. and Kies, M.W.: Protein determination. Biochem. biophys. Acta *45:* 382–384 (1960).

12 Rattazzi, M.; Scandalios, J.G., and Whitt, G.S.: Isozymes. Current topics in biological and medical research, vol. 1 (Liss, New York 1977).

13 Turner, D.C. and Eppenberger, H.M.: Developmental changes in creatine kinase and aldolase isoenzymes and their possible function in association with contractile elements. Enzyme *15:* 224–238 (1973).

14 Vesell, E.S.: Medical uses of isozymes; in Markert, Isozymes, vol. II, pp. 1–28 (Academic Press, New York 1975).

15 Whitt, G.S.: Isozymes and developmental biology; in Markert, Isozymes, vol. III, pp. 1–8 (Academic Press, New York 1975).

16 Wilkinson, J.H. and Steciw, B.: Serum creatine kinase activity. Clin. Chem. *16:* 370–374 (1970).

K. Diane Courtney, Environmental Protection Agency, MD 67, Research Triangle Park, NC 27711 (USA)

Varia

ACPEF Announces Special Research Award

The American Cleft Palate Educational Foundation, Inc. announces its special meritorious research award for professionals who have recently completed or are completing their training. Winners will receive an 'Award of Special Merit'. For more information contact: Dr. Curt Weiss, Department of Communicative Disorders, University of New Mexico, 608 Buena Vista N.E., Albuquerque, NM 87131. Deadline for receiving manuscripts is January 19, 1979.